

ELX-5795L-6 (BXTD 9006.3)
PATENT

A m endments to the Claims:

Claims 1-9 were cancelled in the Preliminary Amendment.

10. (previously presented) A method of expressing recombinant biologically active human erythropoietin comprising the steps of transfecting a host cell line with DNA, RNA, or nucleotide sequence consisting essentially of the Apa I restriction fragment of a human erythropoietin gene, contacting the transfected cells with culture medium to permit the cells to express erythropoietin, and recovering the expressed erythropoietin.

11. (previously presented) The method of Claim 10 wherein the Apa I restriction fragment is carried on a plasmid or virus.

12. (previously presented) The method of Claim 10 wherein the host cell line is selected from the group consisting of eukaryotic cells, yeast and bacteria.

13. (previously presented) In a method of expressing recombinant biologically active human erythropoietin from a cell line in a contact with an incubating medium, the improvement which comprises incorporating in said method a cell line capable of permitting a yield of erythropoietin in the incubating medium, said cell line having been produced by transfecting a host cell line with DNA, RNA, or nucleotide sequence consisting essentially of the Apa I restriction fragment of a human erythropoietin gene.

14. (previously presented) The method of expressing recombinant biologically active human erythropoietin from a cell line in contact with an incubating medium in

ELX-5795L-6 (BXTD 9006.3)
PATENT

accordance with Claim 13, wherein said cell line is capable of permitting a nominal yield of at least two million Units of erythropoietin per liter of incubating medium.

15. (previously presented) The method of Claim 13 wherein said Apa I restriction fragment is carried on a plasmid.

16. (previously presented) The method of Claim 13 wherein said Apa I restriction fragment is carried on a virus.

17. (previously presented) The method of Claim 13 wherein the host cell line is selected from the group consisting of eukaryotic cells, yeast and bacteria.

18. (previously presented) A method of making a glycoprotein exhibiting erythropoiesis regulating activity comprising:

culturing eukaryotic host cells transformed with a DNA construct comprising a eukaryotic promoter sequence operably linked to an insert consisting essentially of the sequence of SEQ ID NO:1 from position 59 through position 2204, the construct also including a 5' untranslated sequence located between the eukaryotic promoter and position 59, the 5' untranslated sequence being devoid of a sequence encoding a translational initiation codon, the construct also including a 3' untranslated sequence downstream of position 2204, the 3' untranslated region comprising a eukaryotic polyadenylation sequence, where the host cells are cultured under conditions providing for the expression and secretion of the glycoprotein into a culture medium; and recovering the glycoprotein from the culture medium.

19. (previously presented) The method of claim 18 in which said eukaryotic host cells are baby hamster kidney cells.

ELX-5795L-6 (BXTD 9006.3)
PATENT

20. (previously presented) The method of claim 19 further comprising transforming the host cells with a DNA sequence encoding dihydrofolate reductase and where the host cells are treated with methotrexate prior to recovering the glycoprotein from the culture medium.

21. (currently amended) The method of claim 20 wherein the host cells are treated with a concentration of methotrexate of about ~~10- μ M~~ ~~1 μ M to about 1 mM~~; and wherein cells that continue to grow after methotrexate treatment are selected to establish a stable cell culture.

22. (canceled)

23. (canceled)

24. (previously presented) The method of claim 18 wherein the DNA construct includes an adenovirus major late promoter as the eukaryotic promoter operably linked to the insert.

25. (currently amended) The method of claim 18 wherein the DNA construct includes a ~~metallothionein~~ metallothionein promoter as the eukaryotic promoter operably linked to the insert.

26. (previously presented) The method of claim 18 wherein titers of said glycoprotein of at least two million units of erythropoietin activity per liter of culture medium are obtained, the units of activity being measured by a radiolimmune assay using a mammalian erythropoietin as a standard.

ELX-5795L-6 (BXTD 9006.3)
PATENT

27. (previously presented) A method of making a glycoprotein exhibiting erythropoiesis regulating activity comprising:

culturing eukaryotic host cells transformed with a DNA construct comprising an insert consisting essentially of the sequence according to SEQ ID NO:1 under conditions providing for the expression and secretion of a glycoprotein into the culture medium; and

recovering said glycoprotein from the culture medium.

28. (previously presented) The method of claim 27 wherein the cells are stably transformed and the glycoprotein is produced at a level of about 500 to about 7000 units per ml of culture medium, the units being determined by an in vitro erythroid colony forming bioassay using mouse bone marrow cells and partially purified sheep erythropoietin as a comparative standard.

29. (previously presented) The method of claim 27 wherein the cells are stably transformed and the glycoprotein is produced at a level of about 6 to about 85 µg per ml of culture medium.

30. (previously presented) The method of claim 27 in which said eukaryotic host cells are baby hamster kidney cells.

31. (currently amended) A method of making a glycoprotein exhibiting erythropoiesis regulating activity comprising:

culturing eukaryotic host cells transformed with a DNA construct comprising an insert consisting essentially of the sequence according to SEQ ID NO:1, an adenovirus adenovirus-2 major late promoter sequence, an adenovirus-2 tripartite leader and third leader 5' splice sequence, an immunoglobulin 3' splice sequence and a late

ELX-5795L-6 (BXTD 9006.3)
PATENT

SV-40 polyadenylation signal sequence, the insert being operably linked downstream of the adenovirus adenovirus-2 major late promoter sequence and upstream of the immunoglobulin 3' splice site to provide for the expression and secretion of the glycoprotein into a culture medium; and
recovering said glycoprotein from the culture medium.

32. (previously presented) The method of claim 29 in which said eukaryotic host cells are baby hamster kidney cells.

33. (currently amended) A method of making a glycoprotein exhibiting erythropoiesis regulating activity comprising:
culturing eukaryotic host cells transformed with a DNA construct comprising an insert consisting essentially of the sequence according to SEQ ID NO:1 and a metallothionein metallothionein promoter operably linked to the insert to provide expression and secretion of a glycoprotein into the culture medium; and
recovering the glycoprotein from the culture medium.

34. (withdrawn) A glycoprotein exhibiting erythropoiesis regulating activity produced by the method of claim 10.

35. (withdrawn) A glycoprotein exhibiting erythropoiesis regulating activity produced by the method of claim 18.

36. (withdrawn) A glycoprotein exhibiting erythropoiesis regulating activity produced by the method of claim 27.

ELX-5795L-6 (BXTD 9006.3)
PATENT

37. (withdrawn) The glycoprotein according to claim 36 wherein the glycoprotein exhibits microheterogeneity in size when analyzed by SDS polyacrylamide gel electrophoresis, and where a first pattern of bands of the glycoprotein detected by Coomassie staining of the SDS polyacrylamide gel comigrates with a second pattern of bands detected when erythropoietin purified from the urine of a patient with aplastic anemia is analyzed by SDS polyacrylamide gel electrophoresis.

38. (withdrawn) A glycoprotein exhibiting erythropoiesis regulating activity produced by the method of claim 31.

39. (withdrawn) A glycoprotein exhibiting erythropoiesis regulating activity produced by the method of claim 33.